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ELECTROLYTE PROFILE OF RABBIT FED WITH *NAPOLEONAE IMPER1ALIS* LEAF EXTRACT.

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ABSTRACT

The effects of ethanol and water extracts *of Napoleonae imperialis* on magnesium trisilicate induced diarrhoea were studied in rabbits by monitoring the ions usually lost in stool when there is diarrhoea. The levels of the ions were measured in sera. The oral administration of 5ml each of ethanol and water extract caused the retention of 2726 ± 50.00 mg/mi and 1454.00 ± 28.00 mg/ml of chloride respectively. With 2ml each of the ethanol and water extracts, 503.00 ± 26.00 mg/ml and 1147 ± 10.80mg/ml of chloride were retained. 5ml and 2ml of the ethanol and water extracts caused about the same quantity of Mg^{2+} (93 mg/ml) to be retained in the sera of the rabbits. The levels of K^{+} retained when 5ml of the ethanol and water extracts were administered were 80.00 ± 0.007 mg/ml and 83.33 ± mg/ml respectively, while the levels retained upon administration of 2ml of the extracts were 8.33 ± 3.33mg/ml and 21.67 ±3.33 mg/ml. When 5ml each of the ethanol and water extracts were given, 20.322 ± 0.581ug and 23.645 ± 1.290 fig of PC>4^{3} were found in the sera, while 2ml of the extracts caused 24.419 ± 2.129 ug and 26.677 ± 0.387 ug of PO_4^{3-} to be retained. The levels of HCO_3'' retained in the sera with 5ml of the ethanol and water extracts were 29.983 ± 1.663 ug and 28.036 ± 1.238 ug each, while 2ml of the caused the retention of 23,508 ± 0.003 ug and

22.489 ± 0.718 ug respectively. Both 5ml and 2ml of the ethanol and water extracts caused almost the same level of Na^+ ions to be retained at approximately 4mg/ml. The results of this study strongly indicate that 5ml of the ethanol extract *of Napoleonae imperialis* had a more effective antidiarrhoeal effect than both 5ml and 2ml of the same extracts.

KEYWORDS: *Napoleonae imperialis,* effective antidiarrhoeal, magnesium trisilicate.

INTRODUCTION

One of the major uses of plants apart from food, clothing, shelter and timber is its therapeutic use. Traditional medicine (TM) can be described as health practices, approaches, knowledge and beliefs incorporating plants, animals, spices and mineral-based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to treat, diagnose and prevent illness and also maintain well-being (WHO, 2006), It has estimated that more than 40% of prescribed medicines today, even in the western world comes directly or indirectly from plants (ASICUMPON, 2000).

Countries in Africa, Asia and Latin America use traditional medicine <TM) to help meet some of their primary health care needs. In Africa, it was estimated that up to 80% of the population uses traditional medicine for primary health care (WHO, 2006). Thus, dependence on medicines from indigenous plant is specially predominant in developing countries where western medicine is often unavailable or simply too expensive (ASICUMPON, 2000).

Traditional medicine (TM) or traditional Africa medicine (TAM) has come a long way from the times of our forefathers,some said that a particular plant was shown to them in dream to cure a particular illness, some will inherit it from either their father or close relatives. It is because of these that Elujeba described traditional Africa Medicine (TAM) as our Socio- economic and socio - cultural heritage, serving over 80% of the population in Africa (Elujoba et al.,2005) .

Herbal Medicine also known as herbalism, herbao%y_y Medical botany, botanical medicine, phytotherapy etc is a traditional medicinal plants and plant exracts. The bioinformatics related to this subject could be referred to as med- referred to as med-Botanics (white and MaCal, 2006. Medicinal plants are plants in which one or more of their parts contains substance that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (W H O, 2000). The Word Health, Organization consultative group that formulated this definition State also that such a description makes it possible to distinguish between Medicinal plants

whose therapeutic properties and constituents have been established Scientifically and plants but which have not yet been subjected to a through scientific Study *(WHO, 1999). It was this that accelerated the use and search for drugs and* dietary supplements derived from plants in these days by a Biochemist, Pharmacologist, Microbiologist, Botanist and Natural products.
In fact, many modern drugs have been derived from plants (Famsworth, 1991).

Various numbers of plants have been used in traditional Medicine for number of years. Quite number of them do cure different illness although there may be no sufficient scientific data (double blind trial, for example) to confirm their efficacy (WHO, 2000). According to Association of Scientific identification, conservation and utilization of medicinal plants of Nigeria (ASICUMPN) there are different Medicinal plants in Nigeria, these plants are sometime implicated in some recipes for the treatment of different server $_t$ diseases in Nigeria. Some of the plants are; Napoleonae imperials for treatment of gonorrhea, Rauwafia Vomitotia for treatment of stomach aches, morinda Lucida for treatment of small pox, piper guineinse that cure rheumatism, Garcunia Kola used for treatment of hypertension, Ocinum basillicum as cure for typhoid fever, Xylopia acthiopica as a remedy *for* convulsion , kalanchoe pinnatum for stomach pains e. t. c. Also some of these plants listed above can cure more than a disease (eg) *Napoleonae imperials* depending on the number of phyto chemical content and also the region using it (ASICUMPN 2000).

Napoleonae imperialis is a medicinal plant that belongs to the family Lecythidseeae which is a small tropical family that grows in all the regions of Nigeria and other parts of west Africa. The plant is commonly known as ntum in the Ikwuano dialect of Ibo language in Nigeria. In Ngbo (my Community it is mostly found in Ojiegbe village. This plant is a small evergreen tree up to *5m* in height, leaves alternate, 8 -12cm.flowers of unusual appearance, Saucer shapped, brown, 3-4 cm across. Fruit a globose drupe. This plant is grown in the garden for its peculiar flowers which arise on woody branches mainly.

Though, *Napoleonae. imperialis* is one of the lesser known plants, its economic importance has been reported by (Irving ,1993). These includes; the use of the fruits sugary pulp as deserts, an infusion of the leaves is used to dissolve clothed blood in freshly delivered women, stem is used to cure gonorrhea, roots are used to fevers and the twigs as traditional chew-sticks. Not much is known of the chemical composition of the leaf,bark and roots have been particularly determined . Not much is known of the chemical composition of the seeds.The investigation therefore shows that the seeds of *Napoleonae mqjerialis* are rich source of commercial haemolytic saponin seeds

extracted from fruit *of Napoleonae imperialis* in Nigeria were processed into a drug powder and analysed for nutrients, calorific value and anti nutritional factor. Ihe mineral contents of the seed meal included 5.01g/kgca,17.5g/kgk and 16.lg/kg Na. while the values for saponin and cyanide contents were 20% 3 5mg/kg respectively.

Moreover, with respects to this topic Electrolyte profile of rabbit fed with Napoleaona imperalis, some of the electrolytes (cations and anions) such as chloride (Cl), Calcium(Ca), Sodium (Na^4) Potassium(K^+) Magnesium(M_g^+), Sulfate,(S) , Phosphate ($HPO4^{2)}$ and Biocerbonate (HcOs'). These electrolytes helps in the management of stomach problems .

Phosphate is mainly an intracellular ion and is seen in all cells. The major function are: formation of bone and teeth, production of high energy phosphate compounds such as ATP, CTP,GTP. Creatinine phosphate e. t. c.

Synthesis of nucleoside and co-enzymes such as NAD and NADP (vasudevan and Sreekumari., 2005). The serum level of phosphate is 3-4 mg/d/ in normal adults and is 5-6 mg/d/ in children (Vasudevan and Sreekumari,.2005). Phosphate level is mainly affected by parathyroid hormone and phosphate transport.

Chloride (CL") constitutes approximately by 0.15% of human body weight. Cl" concentration in plasma is about 96-106meq/l and in cerebros final fluid (CSF) is higher than any other body fluids. The major functions of chlorides includes: formation of hydrochloric acid (HCL) in gastric Juice, it is essential in maintaining the proper acidic environment for pepsin (Vasudevan and Sreekumair;2005). The chloride level is affected by excessive vomiting, dehydration, renal tubular acidosis, severe diarrhea or use of diuretics e. t. c. (Vasudevan and Sreekumari 2005;)

Bicarbonate (HCO3) the most important buffer system in the plasma is the bicarbonate carnonic acid systems ($NaHCO3/H_2Co_3$) bicarbonate level varies from 22-26m mol/liter with an average normal value of 24mmol/liter. Some other physiological importance of bicarbonate are:- it is one of the major buffering system used to maintain the PH of mammalian blood., used in the formation of acid in the lumen on the stomach and used in neutralizing the PH of chyme leaving the Stomach and altering the small intestine (Vander,1990). The level of HCO3 in plasma B affected by the pancreatic secretion of HC03 , prolonged diarrhea, womiting e .t. c.

AIMS/OBJECTIVE

This research work investigated the Electrolyte profile of Rabbit fed *wtthNapoleonae imperialis* leaf extracts.

MATERIALS AND METHOD

BIOLOGICAL MATERIALS

The following materials were used

- Five (5) rabbits (experimental animals)
- Dried leaves *ofNapoleonae imperialis*
- « Vital feed (grower's mash)

METHODS

COLLECTION OF BIOLOGICAL MATEMALS

DRIED LEAVES

The leaves of *Napoleonae imperialis* collected from Ojiegbe, Amogu in Ameku Autonomous community, Ngbo Central Development Centre of Eboyi State and allowed to dry.

ANIMALS (RABBITS)

The five (5) rabbits of both sexes were obtained from animal house in the *Zoology* Department, University of Nigeria Nsukka (UNN). The animals were acclimatized for one (1) week (7 days) and maintained on their normal diets. The animals were divided into three (3) groups comprising two (2) in group A, B and one (1) in group C which is the control, i.e. *AZ, 82* and *C\.*

GRINDING AND EXTRACTION OF LEAVES.

The homogenation (grinding) of the-dried leaves was done **with** grinding machine while the **extraction of leaves was done in** ethane! and water.

EXTRACTION IN ETHANOL

93. 0g of the homogenized(ground) **leaves** of *Napoleonae imperials* was -soaked in 200ml of ethanol and allowed **to stand** overnight. It was filtered the following morning to obtain the ethanol **extract** after which 50ml of the extract was concentrated by mild heading on a hot **plate** to obtain a jelly-like residue WiiiCii WHS COucCtcu Suu put ImG *a* COntSIficf Sfiu StOTCu In E rGiJigeiatOr tO uc used later.

EXTRACTION IN WATER

96.0 of the homogenized (ground) leaves **of *Napoleonae* *imperials*** was soaked in 200ml of distilled water and allowed to **stand overnight,** it was filtered to obtain the aqueous extract after which **50ml of the extract** concentrated by mild heating on a hot plate to obtain a jelly-like **residue which** was collected and stored in a refrigerator for use.

MEASUREMENTS OF WEIGHT OF ANIMALS

The weight of the animals were measured to be able to determine the quantities of extract per kg body administered.

INDUCING FOR DIARRHEA

Magnesium trslilicate (an antacid) was used to induce diarrhoea in the rabbits. The drug which was in tablet form was crushed two (2) tables to powdered form and was mixed with 100ml of distilled water and stirrer very well to ensure thorough mixing.

ADMINISTRATION OF DRUG.

Oral administration was used. Magnesium trisilicate was administered orally. The- animals were administered the extracts according to their individual weights, it was administered organic and aqueous extracts of *Napoleonae imperials* orally at doses of 5ml and 2ml respectively daily for five (5) days. 3.3.2

COLLECTION OF SPECIMEN

Blood from animals (Rabbits): The rabbits were starved of food and water for 24 hours (a day) after the five (5) days of feeding and administration of the extracts. Subsequently, the blood samples were collected from the rabbits from the ear veins with syringes into venoject tubes containing EDTA anticoagulant.

PREPARATION OF SERUM

The blood was centrifuged at 3000xg for lOminutes and allowed to stand. The supernatant (serum) was collected using micro-pipette and transferred into five (5) test tubes and stored in the refrigerator for use.

DETERMINATION OF SERUM PHOSPHATE

Method

Six test tubes were labeled A, B, C, D, E and F. Test tube A contained O.lml of 5% ethanol extract, test tube B contained O.lml of 2.5% ethanol organic extract, test tube C contained O.lml of

5% aqueous extract, test tube D contain O.lml of 2.5% aqueous extract, test tube E contained O.lml of the sample (serum) which served as the control while test tube F was the blank. To each of these test tubes except the blank test tube were added 0,1ml of the sample(serum), 4.9ml of water(distilled), 1.5ml of vanadium molybdate,lml of ammonia solution and 2 drops of nitric acid and made up to 10 mi with distilled water, was shaken very well and lastly, the absorbance was read at 480nm.

CHLORIDE Procedure

To five different test tubes were added 0.1ml of the sample (serum), 0.9ml water, 0.2ml of concentrated nitric acid and was heated on hot plate under the fume chamber. A few drops of potassium permanganate solution were added warmed again for 2 minutes, and 2ml of ferric alum solution was also added and followed with 1ml of alcoholic thiocyanate.

DETERMINATION OF SERUM CHLORIDE

Method: To each of the five different test tubes, the following were added: 0,1ml of the serum, ethanol extracts 5%, 2.5% and aqueous extracts 5%, 2.5% and O.ml of the control respectively and was added 0.9ml of distilled water, 0.2ml of concentrated nitric acid and was heated until the colour changes to pale yellow, then added again a few drops of potassium permanganate solution and worm again for 2 minutes under the fume chamber and 2ml of ferric alum solution plus 1ml of alcoholic thiocyanate was added and was shaken very well, then the absorbance reading was taken at 470nm.

MAGNESIUM

Procedure: O.lml of the sample (serum), 5.9ml of distilled water,2ml of 10% sodium tungstate, 2/3 normal sulfuric acid were added to each of the five test tubes and centrifuge for 5minutes, 5ml of the supernatant were collected and 1ml of 0.05% tetan yellow was added then 2ml of 4 normal sodium hydroxide.

DETERMINATION OF SERUM MAGNESIUM

Method: To each of the five different test tubes which contain O.lml of the serum *I* of organic extract (Ethanol) 5%, 2.5% and aqueous(distilled water) extracts 5%, 2.5% and O.1ml of the control respectively and was added O.lml of the control respectively and added 5.9ml of distilled water, 2ml of 10% sodium tungstate and 2/3 normal sulfuric acid and centrifuge for 5minutes, 5ml of the supernatant was collected and 1ml of 0.05% tetan yellow plus 2ml of 4 normal sodium hydroxide which was thoroughly mixed and take the absorbance reading at 520nm.

POTASSIUM

Procedure: O.lml of the sample, 0.9ml of distilled water, 2ml of sodium cobalt nitrite reagent, then shake slowly and continuously for 2 minutes, allow to stand for 45minutes then add 2ml of distilled water, mixed the mixture very well and centrifuge for 15mins, Drain off the supernatant and add 1ml of H20 to the residue, shake for 2 minutes and centrifuge for 5 minutes. Run off the supernatant again and add 2ml of 70% ethanol to the residue and shake for 2minutes, centrifuge for 5minutes and after that run off the supernatant again and add 2ml of distilled water and shake frequently and allow to cool, then add 1ml of choline hydrochloride then 1ml of 2% sodium ferry cyanide and make up to 6ml with water.

DETERMINATION OF SERUM POTASSIUM Method

To each of the five test tubes was added 1ml of serum of the organic extract (ethanol) 5%, 2.5% and aqueous extract (water) *5%*, 2.5% and of the Control. Also, 0.9ml of distilled water, 2ml of sodium cobalt nitrite reagent was added to the five test tubes and was shake slowly and continuously for 2minutes. Allow to stand for 45mins, then add 2ml of distilled H2 which was mixed thoroughly and was centrifuge for ISminutes, drain off the supernatant and add 1ml of water to the residue, shake for 2minus and centrifuge for 5mins, run off the supernatant again and add 2ml of 70% ethanol to the residue and boil for 10 Minutes , shake frequently and allow to cool then add ims of choline hydrochforide and 1ml of 2% sodium ferry cyanides and make up to 6ml with H2° and take the absorbance at 62onm. Blank preparation; 2ml of distilled water, 1ml of 1% of choline hydrochloride.

DETERMINATION OF SERUM SODIUM

Method: To each of the five different tubes containing 0.1 ml of the sample (serum) respectively was added 4.9ml of li?^{0}, 5ml of zinc uranyl acetate and was allow to stand for 5minutes and then centrifuge for 3Qmms and discard. Then add 8ml of 1% of acetic acid, 0.4ml of 10% of potassium ferrycyanide to the supernatant and was allow to stand for lOmunites. At the end of these processes, the mixture turn pale blue and the absorbance was taken at 480nan.

Procedure: 1ml of the sample, layer it with paraffin, add 20ml of saline phenol red solution under paraffin. Into another tube, 1ml of the sample, 5ml of O.Olnormal Hcl, sware it for 2minutes, then pour the solution into the test tube similar to the one already use. Add lOmls of 0.9% of sodium chloride, 7 drops of phenol red solution then titrate with 0.01 normal sodium hydroxide until the colour matches the other one under paraffin.

DETERMINATION OF SERUM BICARBONATE

Method: To each of the five test tubes were added O.lml serum of the organic extract Cethanol) 5%, 2.5% and aaueous solution extract (H?0) 5%, 2.5% and control. To each of those test tubes containing the serum was added 20ml of saline phenol red solution under paraffin, also into another test tube was added 1ml of the sample (serum), 5rnl of O.Olnorrnal HC! and sware for 2 minutes then pour the solution into the test tube similar to the one already in use, add 10ml of 0.9% of sodium chloride, 7 drops of phenol red solution and titrated with O.Olnormal sodium hydroxide until the colour matches the other one under paraffin. At the end of the mixture, the sample turns into pale blue in colour and the absorbance reading was taken at 720nm. Blank preparation: A blank titration of 5ml of 0.01 normal HC1 plus 10ml of 0.9% of sodium chloride and 7 drops of phenol red solution was used.

4. O RESULT

Table 4.1, Yield Of Ethanol And Water Extracts Of *Navoleonac imverialis.*

Mass of leaves before extraction (g)	Mass of leaves After extraction(g) in ethanol	Mass of extract	Volume of Extract administered	Percentage yield of extracts
Organic extract - 140.00	85.9	54.10	2ml	38.64%
Water extract -100.00	Aqueous=80	20.00	5ml	20% -

The percentage yields from the table above of ethanol and aqueous extracts are 38.64% and 20% respectively. These shows that more of the chemical components of *Napoleonae imperialis* are more soluble in ethanol than aqueous extracts.

4.1 PARAMETERS

ELECTROLYTE CONCENTRATIONS OF SERUM THE SAMPLE

4.2 CHLORIDE

Table 4:2

Extract	Sample concentrations (mg/ml)	
Ethanol	5ml extract	2ml extract
	2726-OOt 50.00	503.00±26.00
Water	1454.00128.00	1147.00±10.80
Control = 846.00±88.00		

Ethanol extracts had a higher anti-diarrhae effect than water extract

4.3 MAGNESIUM

Table 4:3

Extract	Sample concentrations (mg/lOOml)	
Ethanol	5ml extract	2ml extract
	93.027+ (X 001	93.146+0,001
Water	93.240± 0.001.	93.228± 0.002
Control = 67.568±0.002		

Here, ethanol and water extracts had almost the same of anti-diarrhea effects.

4.4 POTASSIUM

Table 4:4

Extract	Sample concentrations (mg/ml)	
Ethanol	5ml extract	2ml extract
	80.00+ 0.007	8.33±3.33
Water	83.33+3.33	21.67± 3.333
Control =11.667+3.333		

In potassium, the water extract had a higher anti-diarrhea effect than ethanol extracts

4.5 PHOSPHATE

Table 4:5

Extracts	Sample concentrations (f-ig)	
Ethanol	5ml extract	2ml extract
	20.322± 0.581	24.419 ±2. 129
Water	23.645± 1.290	26.677+0.387
Control = 28.7o9±3.548		

In phosphate, organic extract has a lower anti-diarrhea than water extract.

4.6 BICARBONATE

Table 4:6

Extracts	Sample concentrations (jig)	
Ethanol	5ml	2ml
	29.983±1.663	23.508 ±0.063
Water	28.036±1.238	22.489± 0.718
Control = 18.9311 1.319		

In bicarbonate, organic extracts has a higher anti-diarrhea effect than water extract.

4.7 **SODIUM**

Table 4:7

Extracts	**Sample concentrations (mg/ml)**	
Ethanol	5ml	2ml
	4.532+0.012	4.376± 0.012
Water	4.597 ±0.025	1.603± 0.012
Control - 8.63 1± 0.025		

Here, ethanol and water extracts had almost the same effect in lowering the concentration of the ion.

DISCUSSION

The extraction of dried leaves of ***Napoleonae imperialis*** with aqueous and organic extracts yielded 38.64% and 20% respectively. These values suggest that most of the chemical components of the leaves are only slightly extractable with ethanol. Also, a drug- induced diarrhea was also administered at the volume of 1 .0ml which contains 0.02ml concentration of the tablet (drug). But the drug was administered in excess, which 2.0ml was administered instead of 1 .0ml.

The volume of extracts administered to the rabbits were 2.oml and 5.0ml of organic (ethanol) and aqueous respectively. On the course of extracts administration, the following electrolyte anti-diarrhea concentration effects were obtained with 2.0.ml ethanol extract was however reduced significantly compared with the control as seen in the tables of chapter four, 4.376 ± 0.012 mg/100ml for Na^+, 8.333 ±3.333 mg/ml for k^+, 24.419 ± 2.129 for po_4^{2}, 23.508 ± 0.063[1g for **$HCO3^-$,** 503.00 ±26.00 mg/ml for CL^- and 93.146 ±0.00 mg/100ml for mg^{2+} respectively.

Accordingly trial with 5.0.ml ethanol extract yields 4.522 ± 0.012 mg/100ml, 80.00 ±0.00 1 mg/ml, 20.322 ±8.581ug, 29.930+1. 663 ug, 2726.00 +50.001 mg/ml and 93.027 ±0.001 mg/100ml respectively.

Generally, in organic extract administration both with 2. Oml and 5.0ml, the yielded extract concentration of electrolyte anti-diarrhea effects occurs in this order CL^- > mg^{2+}> po_4^{2-}-> $HC0_{3-}$> K^+ > Na^+ while in aqueous extract concentration of anti-diarrhea effects occurs in this order, for 2.0ml gives CL^- >mg^{2+} > Po_4^{2}-> HCo_3^-> K^+> Na^+ and 5.0ml gives CL^-> mg^{2+}> K^+>Po_4^{2}->Hco_3->Na^+.

CONCLUSION

Napoleonae imperials is an active anti-diarrhea, this was observed/manifested in this research work, during the period of this researcher to know really **if Napoleonae imperoalis** is anti-

diarrhea, a drug know as magnesium trilisicate antacid was injected/induced to the rabbit to stimulate the diarrhea effect on the rabbit but due to fact that **Napoleonae imperialis** is an active anti-diarrhea leaf no diarrhea effect was seen on the rabbit the period given (5days), this it as a result of the **Napoleonae imperialis** extract administered to the rabbit from this my observation I want to request that more effort are to put by medicinal -herbalist to identify **Napoleonae imperials** as one of the effective medicinal plant that have anti-diarrhea properties in them. I also want to appeal with those involved in research on medicinal plants and screening plants for bioactive agent to put more efforts in their biological screening with **Napoleonae imperialisto** find out all biochemical properties embedded in **Napoleonae imperialis** that makes it Ethno medicinal.

Finally, organic extract has a higher concentration of anti-diarrhea effect than aqueous extracts.

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